Reevaluating Fluoroquinolone Breakpoints for *Salmonella enterica* Serotype Typhi and for Non-Typhi Salmonellae

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Salmonella enterica **infections cause considerable morbidity and mortality worldwide. Antimicrobial therapy may be lifesaving for patients with extraintestinal infections with *S. enterica* serotype Typhi or non-Typhi salmonellae. Because antimicrobial resistance to several classes of traditional first-line drugs has emerged in the past several decades, the quinolone antimicrobial agents, particularly the fluoroquinolones, have become the drugs of choice. Recently, resistance to nalidixic acid has emerged among both Typhi and non-Typhi *Salmonella* serotypes. Such *Salmonella* isolates typically also have decreased susceptibility to fluoroquinolones, although minimum inhibitory concentrations of the fluoroquinolones usually are within the susceptible range of the interpretive criteria of the NCCLS. A growing body of clinical and microbiological evidence indicates that such nalidixic acid–resistant *S. enterica* infections also exhibit a decreased clinical response to fluoroquinolones. In this article, we recommend that laboratories test extraintestinal *Salmonella* isolates for nalidixic acid resistance, we recommend that short-course fluoroquinolone therapy be avoided for infection with nalidixic acid–resistant extraintestinal salmonellae, and we summarize existing data and data needs that would contribute to reevaluation of the current NCCLS fluoroquinolone breakpoints for salmonellae.**

BACKGROUND

Typhoid fever is an acute, generalized infection of the reticuloendothelial system caused by *Salmonella enterica* subspecies *enterica* serotype Typhi that is estimated to cause 16 million illnesses and 600,000 deaths worldwide annually [1]. Non-Typhi serotypes of *S. enterica* are estimated to cause ~1,412,000 illnesses and 600 deaths annually in the United States alone [2]. Timely treatment with appropriate antimicrobial agents is important for reducing the mortality of extraintestinal infections due to *S.* Typhi and non-Typhi serotypes [3]. Unfortunately, resistance to traditional first-line antimicrobial agents, such as ampicillin, chloramphenicol, and trimethoprim-sulfonamide combinations, has emerged worldwide among both *S.* Typhi [4–8] and non-Typhi salmonellae [9]. Consequently, fluoroquinolones (e.g., ciprofloxacin), which have been available since the 1980s, have become the mainstay of therapy for invasive salmonellosis [10]. Nalidixic acid is the prototype quinolone. It has been available in many countries since the mid-1960s, but it is now seldom used because of the increasing prevalence of nalidixic acid–resistant salmonellae.

The NCCLS sets standards for antimicrobial susceptibility testing methods and interpretive criteria for the United States; NCCLS recommendations also have considerable influence in many other countries. The current MIC breakpoints for Enterobacteriaceae (including *S. enterica*) for ciprofloxacin are ≥ 4 μg/mL (resistant) and ≤ 1 μg/mL (susceptible). The breakpoints for nalidixic acid are ≥ 32 μg/mL (resistant) and ≤ 16 μg/mL (susceptible) [11]. Although ciprofloxacin-resistant isolates of *S.* Typhi [12] and non-Typhi salmonellae [13–15] have been reported, salmonellae that are ciprofloxacin susceptible and nalidixic acid resistant are currently more prevalent and are increasingly isolated from humans and from food animals [13,

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16–23]. The MICs of ciprofloxacin for these nalidixic acid–resistant isolates are usually increased, although they are still within the current NCCLS range for susceptibility (i.e., 0.12–0.5 μg/mL). Unfortunately, however, reports indicate that patients with extraintestinal nalidixic acid–resistant *S.* Typhi or non-Typhi *Salmonella* infections are less likely to respond adequately to fluoroquinolone therapy than are patients with nalidixic acid–susceptible *Salmonella* infections [17, 23–45]. Such reports suggest that current NCCLS breakpoints for ciprofloxacin may not accurately predict clinical response to treatment of patients with extraintestinal salmonellosis [46]. Here, we review existing evidence and data needs that may contribute to the reevaluation of the NCCLS breakpoints for fluoroquinolones among *Salmonella* species to reflect more accurately the clinical response to therapy.

EPIDEMIOLOGY OF SALMONELLAE WITH DECREASED SUSCEPTIBILITY TO FLUOROQUINOLONES

To determine the antimicrobial resistance patterns of *S.* Typhi isolates, the Foodborne and Diarrheal Diseases Branch of the United States Centers for Disease Control and Prevention (CDC; Atlanta, GA) initiated laboratory-based surveillance for the 1-year period from 1 June 1996 through 31 May 1997 [10]. During this period, state public health laboratories forwarded *S.* Typhi isolates from clinical laboratories to the CDC. Antimicrobial susceptibility testing was performed on all isolates, and a standard questionnaire was administered to patients. In 1996, the National Antimicrobial Resistance Monitoring System [13] was established (http://www.cdc.gov/narms/). Participating state and local health departments forward every tenth non-Typhi *Salmonella* isolate and, since 1999, every *S.* Typhi isolate to the CDC for antimicrobial susceptibility testing for nalidixic acid, ciprofloxacin, and other antimicrobial agents with use of limited-range broth microdilution panels (Sensititre; TREK Diagnostic Systems), in accordance with NCCLS standards and interpretive criteria.

In 1996–1997, 20 (6.8%) of 293 *S.* Typhi isolates reported to the CDC were nalidixic acid resistant [10]. By 2000, the proportion of *S.* Typhi isolates identified through NARMS to be nalidixic acid resistant increased to 41 (23.2%) of 177 isolates. Because ~80% of *S.* Typhi infections reported in the United States are acquired abroad, these data largely reflect the increase of nalidixic acid resistance among *S.* Typhi globally [10]. Because humans are the only reservoir for *S.* Typhi, and because transferable nalidixic acid resistance is uncommon, the emergence of nalidixic acid–resistant *S.* Typhi isolates is, at least in part, the consequence of treatment of patients who have typhoid fever with quinolones, particularly fluoroquinolones. A similar increase in the prevalence of nalidixic acid resistance has been noted among non-Typhi *Salmonella* isolates [47]. In 1996–1997, 16 (0.6%) of 2627 non-Typhi salmonellae tested were resistant to nalidixic acid; by 2000, 34 (2.5%) of 1378 non-Typhi *Salmonella* isolates tested were resistant to nalidixic acid [13]. Unlike *S.* Typhi infections, most non-Typhi *Salmonella* infections in the United States have food animal (e.g., chicken, cattle, swine, or turkey) reservoirs and are acquired domestically. It is likely that the increased prevalence of nalidixic acid resistance among non-Typhi salmonellae that infect humans in the United States is, in part, a consequence of the administration of fluoroquinolones to food animals [48–51].

THE MOLECULAR BASIS OF QUINOLONE RESISTANCE

Bacteria most commonly develop resistance to quinolones by nontransmissible, spontaneously occurring point mutations in chromosomal genes *(gyrA*, *gyrB*, *parC,* and *parE).* These point mutations alter the enzymes (DNA gyrase and topoisomerase IV) that are targets for quinolone drugs. Although altered permeability of bacterial cell membranes [52, 53] and efflux pumps are not well understood, these mechanisms also play a role in quinolone resistance for some isolates and are not known to be transmissible [54, 55]. More recently, a multidrug-resistance plasmid was discovered [56] that encodes transferable resistance to quinolones via the *qnr* gene. The *qnr* gene product has been demonstrated to directly protect DNA gyrase from quinolone inhibition [57].

Chromosomal point mutations resulting in alterations of the A subunit of DNA gyrase that lead to quinolone resistance have been defined in a substantial number of clinical and laboratory isolates of Enterobacteriaceae, including *Escherichia coli* [58]. These alterations of the target enzyme are clustered between amino acids 67 and 106 in the amino terminus of the A protein known as the quinolone resistance–determining region [59]. Similar chromosomal mutations and changes in the A subunit have been documented for isolates of *S. enterica* [14, 38, 60]. Single chromosomal point mutations have been demonstrated to be sufficient to cause an amino acid change and to result in nalidixic acid resistance. Two or more chromosomal point mutations are usually necessary to result in ciprofloxacin resistance, on the basis of current NCCLS interpretive criteria [54].

DISTRIBUTIONS OF MICs OF QUINOLONE AMONG SALMONELLAE

It is important to consider how antimicrobial susceptibility testing might be used to better predict the clinical outcomes

for patients with extraintestinal salmonellosis treated with fluoroquinolones. To examine this, we prepared scatterplots of MICs of nalidixic acid and compared them with those of ciprofloxacin for *S.* Typhi (figure 1) and for non-Typhi salmonellae (figure 2) submitted to NARMS for 1999–2000 and 1996–2000, respectively [13]. Current NCCLS breakpoints for ciprofloxacin (resistant, ⩾4 μg/mL; susceptible, ⩽1 μg/mL) and for nalidixic acid (resistant, ⩾32 μg/mL; susceptible, ⩽16 μg/mL) are marked in both figures. For both *S.* Typhi and for non-Typhi salmonellae, MIC distribution curves for nalidixic acid are bimodal, with modal peaks at ⩽4 μg/mL and 256 μg/mL. However, it is not possible to clearly differentiate 2 populations using the MIC data for ciprofloxacin. Nonetheless, nalidixic acid–resistant salmonellae tend to have MICs of ciprofloxacin that cluster within the upper part of the current susceptibility range (0.12–0.5 μg/mL), whereas nalidixic acid–susceptible salmonellae tend to have MICs of ciprofloxacin of ⩽0.03 μg/mL (figures 1 and 2). On the basis of these data, testing *Salmonella* isolates for nalidixic acid susceptibility would appear to be a useful screening test for reduced susceptibility to fluoroquinolones. A screening test using nalidixic acid disks has been evaluated and demonstrates high sensitivity and specificity for detecting salmonellae with reduced susceptibility to ciprofloxacin (MIC, ⩾0.125 μg/mL) [61]. However, outliers can be seen on our scattergrams (figures 1 and 2), indicating that the nalidixic acid screening test has some limitations.

CLINICAL AND BACTERIOLOGICAL RESPONSE OF *SALMONELLA* INFECTIONS WITH DECREASED SUSCEPTIBILITY TO FLUOROQUINOLONES

Evidence concerning both the clinical and the bacteriologic response of patients with extraintestinal salmonellosis due to nalidixic acid–resistant *S.* Typhi and non-Typhi salmonellae is available from studies involving laboratory animals or infected patients.

Animal models. *S. enterica* serotype Typhimurium infection of mice is frequently used as an animal model for typhoid fever of humans. The correlation between the MIC and the effective dose of 50% (ED_{50}) of ciprofloxacin for strains of *S.* Typhimurium Definitive Type 104 (DT104) has been studied in the mouse peritonitis/sepsis model. Investigators found that minor changes in the MICs of ciprofloxacin (range, 0.023–0.190 μg/mL), even when remaining within the NCCLS breakpoint for susceptibility, induced major changes in the ED_{50} in the mouse peritonitis model to more than the acceptable dosing range (range, 27–85 mg/kg) [62]. The findings suggest that ciprofloxacin treatment may not be effective for serious *Salmonella* infection when the organism has reduced susceptibility to ciprofloxacin within the current NCCLS susceptible range, as is seen with nalidixic acid–resistant salmonellae [62].

***Human* S. *Typhi* infection.** Since the early 1990s, reports

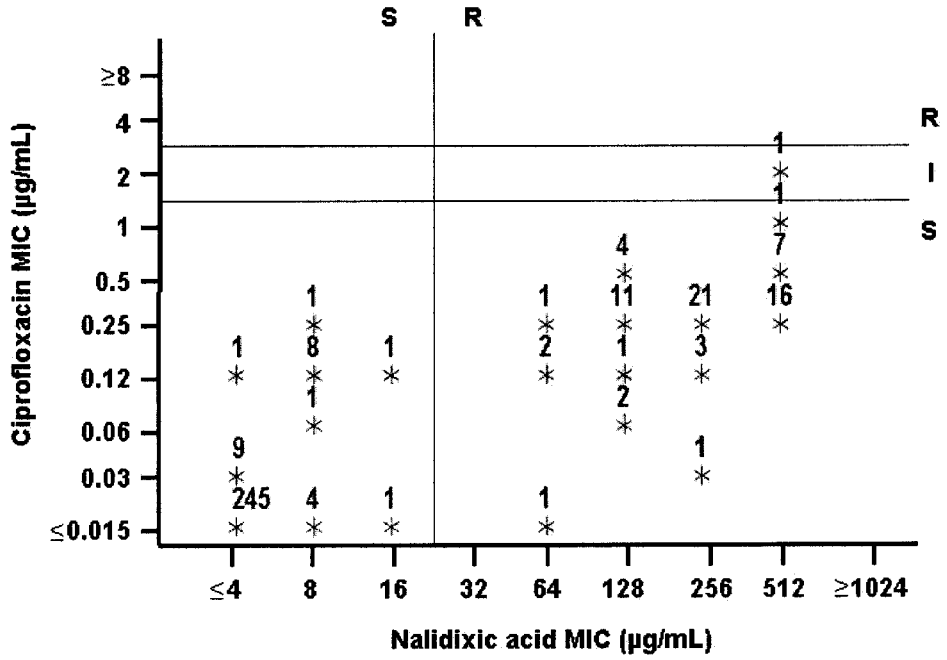

Figure 1. MIC scatterplots for nalidixic acid versus ciprofloxacin for *Salmonella enterica* serotype Typhi, National Antimicrobial Resistance Monitoring System, 1999–2000 (343 *Salmonella* isolates). I, intermediate resistance; R, resistant (current NCCLS breakpoints for resistant organisms are ⩾32 μg/mL for nalidixic acid and ⩾4 μg/mL for ciprofloxacin); S, susceptible (current NCCLS breakpoints for susceptible organisms are ⩽16 μg/mL for nalidixic acid and ⩽1 μg/mL for ciprofloxacin).

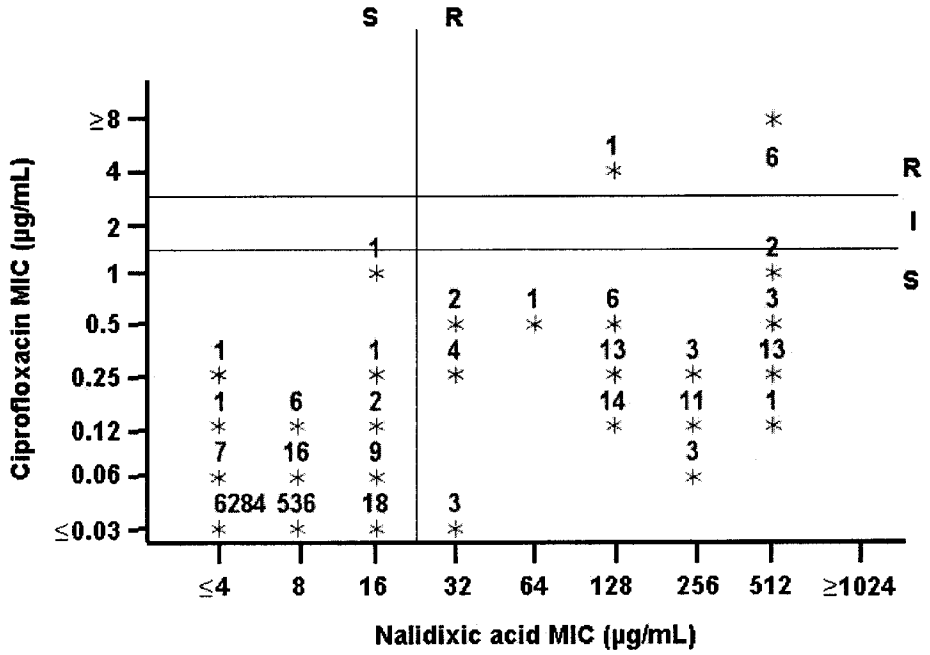

Figure 2. MIC scatterplots for nalidixic acid versus ciprofloxacin for non-Typhi salmonellae, National Antimicrobial Resistance Monitoring System, 1996–2000 (6968 *Salmonella* isolates). I, intermediate resistance; R, resistant (current NCCLS breakpoints for resistant organisms are ⩾32 μg/mL for nalidixic acid and ⩾4 μg/mL for ciprofloxacin); S, susceptible (current NCCLS breakpoints for susceptible organisms are ⩽16 μg/mL for nalidixic acid and ⩽1 μg/mL for ciprofloxacin).

have been published documenting human nalidixic acid–resistant *S.* Typhi infections that did not respond to ciprofloxacin therapy, despite the organisms having MIC values within the susceptible range [16, 17, 26–35, 60]. In 1997, these observations made in case reports were extended by a typhoid fever treatment trial of ofloxacin, a fluoroquinolone with properties similar to those of ciprofloxacin. The study of short-course (2–3-day) ofloxacin therapy conducted in Vietnam for uncomplicated typhoid fever included 117 patients infected with multiple-drug–resistant *S.* Typhi. Of these 117 patients, 99 (85%) were infected with nalidixic acid–susceptible isolates, and 18 (15%) were infected with nalidixic acid–resistant isolates. All *S.* Typhi isolates had MICs of ofloxacin of ⩽1 μg/mL. The median time to fever clearance was 156 h (range, 30–366 h) for patients infected with nalidixic acid–resistant *S.* Typhi and 84 h (range, 12–378 h) for those infected with nalidixic acid–susceptible *S.* Typhi ($P < .001$). Furthermore, 6 (33%) of 18 nalidixic acid–resistant *S.* Typhi infections required re-treatment, whereas 1 (0.8%) of 132 infections caused by susceptible strains required re-treatment (relative risk, 44; 95% CI, 56–345). The authors of this report recommended that short courses (<5 days) of fluoroquinolone therapy not be used for patients infected with nalidixic acid–resistant *S.* Typhi. They also noted that nalidixic acid–resistant *S.* Typhi infections had unsatisfactory responses to treatment with a full 7–10-day course of ofloxacin [60].

Human non-Typhi* Salmonella *infection. The first reports of treatment failures associated with infection due to nalidixic acid–resistant non-Typhi salmonellae (for which the MICs of fluoroquinolone were within the susceptible range) were also published during the 1990s [20, 25, 36, 37, 39–45, 63]. In an outbreak of infection with multidrug-resistant *S.* Typhimurium DT104 caused by contaminated pork that occurred in Denmark during 1998, ciprofloxacin therapy lacked clinical effect for 5 (19%) of 27 patients. Three patients had persistent diarrhea, despite receipt of ciprofloxacin therapy. Two patients died with intestinal perforations, despite receipt of ciprofloxacin therapy at recommended doses. The outbreak strain was resistant to nalidixic acid but had MICs of fluoroquinolone of 0.06–0.12 μg/mL. Such isolates would be considered susceptible to fluoroquinolones, according to current NCCLS MIC breakpoints [20].

In 2002, observations made from case reports were supplemented by data from a matched cohort study of the Danish population. By linking data from the Danish Surveillance Registry for Enteric Pathogens with the Civil Registration System and the Danish National Discharge Registry, 2-year death rates among 2047 patients with *S.* Typhimurium infection were compared with those for a matched sample from the Danish general population. Through their matching criteria, the authors of this study controlled for differences in comorbidity in an effort to account for the potential association between underlying dis-

ease and previous exposure to antimicrobial agents. Patients infected with nalidixic acid–resistant isolates were 10 times (95% CI, 3.3–51.9) more likely to die in the 2 years after infection than were persons in the general Danish population, whereas patients infected with isolates that were resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline but not to nalidixic acid were only 4.8 times (95% CI, 2.2–10.2) more likely to die. Because ciprofloxacin is standard therapy for extraintestinal salmonellosis in Denmark, these data provide strong corroborating evidence that infections with nalidixic acid–resistant non-Typhi salmonellae with an MIC of ciprofloxacin within the susceptible range respond poorly to ciprofloxacin therapy, compared with infections with nalidixic acid–susceptible isolates [25].

PHARMACOKINETIC AND PHARMACODYNAMIC (PK/PD) CONSIDERATIONS

Ratios of serum peak antimicrobial concentration to MIC (peak/MIC) and ratios of 24-h area under the serum concentration-versus-time curve (AUC) to MIC are the major PK/PD determinants of activity for fluoroquinolones [64, 65]. Twenty-four–hour AUC/MIC ratios of $\geqslant$100 are required to produce survival rates approaching 100% in experimental animal infections [66], and AUC/MIC ratios of $\geqslant$125 have been associated with satisfactory outcome in clinical trials of fluoroquinolones among seriously ill patients [67]. Peak/MIC ratios of 8–10 have been shown, both in vitro and in vivo, to prevent the emergence of resistant mutants during fluoroquinolone therapy [68, 69]. These AUC/MIC and peak/MIC ratios are not met for salmonellae with reduced susceptibility to fluoroquinolones (e.g., MIC of ciprofloxacin, 0.5 μg/mL) when treated with standard oral adult doses of ciprofloxacin (i.e., 500 mg twice per day), which may produce serum concentrations of ~2.4 μg/mL and a 24-h AUC of ~23 h $\cdot$ μg/mL [70]. In this example, the peak/MIC ratio would be 5, and the AUC/MIC ratio would be 46. Therefore, predictions from PK/PD data are consistent with observed increased clinical failure rates among persons infected with salmonellae with reduced susceptibility to fluoroquinolones.

DISCUSSION

Considerable data have now accumulated to suggest that infections due to *S.* Typhi and non-Typhi salmonellae with reduced susceptibility to fluoroquinolones may not respond satisfactorily to therapy with ciprofloxacin or other fluoroquinolones, despite MIC values in the current NCCLS range for susceptibility. The findings are consistent with increased clinical failure rates previously observed among persons with *Neisseria gonorrhoeae* infection with decreased susceptibility to fluoroquinolones [71]. Spontaneous chromosomal mutations, selective pressure by use of antimicrobial agents in animals and humans, the potential for clonal expansion of nalidixic acid–resistant salmonellae [72], and the recent discovery of transmissible resistance [57] indicate that quinolone-resistant *Salmonella* infection is likely to become a greater global public health problem.

As might be anticipated, the failure of treatment was identified first for nalidixic acid–resistant *S.* Typhi infections treated with short-course (<5-day) fluoroquinolone therapy [60]. Several studies conducted before the widespread emergence of nalidixic acid–resistant *S.* Typhi demonstrated that fluoroquinolone treatment courses as short as 2 days were >90% effective for treating patients with mild-to-moderate typhoid fever [73–76]. The results of these studies led to wide adoption of short-course treatment strategies to minimize the likelihood of adverse events associated with fluoroquinolone use in children [77], to reduce cost, and to improve patient compliance. There is sufficient evidence in the literature to now recommend discontinuation of short-course fluoroquinolone therapy for extraintestinal nalidixic acid–resistant *S.* Typhi and non-Typhi *Salmonella* infection. There is also some evidence to suggest that standard long-course (7–10-day) fluoroquinolone therapy is less effective for nalidixic acid–resistant *S.* Typhi and non-Typhi *Salmonella* infection.

Additional data are needed to more thoroughly evaluate new fluoroquinolone MIC breakpoints for salmonellae. A better understanding of pharmacodynamics of nalidixic acid–resistant bacteria is needed. It would be useful to investigate clinical response to therapy of *Salmonella* isolates that are nalidixic acid susceptible but have reduced susceptibility to fluoroquinolones (figures 1 and 2). The correlation between the fluoroquinolone disk test zone size and the MIC needs to be further explored to provide data to inform reevaluation of zone size breakpoints for fluoroquinolones. Rigorous studies are needed to determine whether standard courses (7–10 days) and higher doses of various fluoroquinolone class members could reduce clinical and bacteriologic failure rates for extraintestinal nalidixic acid–resistant *S.* Typhi and non-Typhi salmonellae. At present, fewer data are available on the clinical importance of infections due to nalidixic acid–resistant non-*Salmonella* genera of Enterobacteriaceae than for *S. enterica.* However, the evidence that has accumulated for *S. enterica* should also increase research attention to fluoroquinolone breakpoints for other genera of Enterobacteriaceae.

The NCCLS has recently adopted new language advising physicians and laboratories that fluoroquinolone-susceptible strains of *Salmonella* that are determined to be resistant to nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with extraintes-

tinal salmonellosis. The NCCLS advises that testing of extraintestinal *Salmonella* isolates for nalidixic acid resistance may be considered [11]. Moreover, outliers noted on the NARMS nalidixic acid versus fluoroquinolone MIC scatterplots (figures 1 and 2) indicate that this screening test will not identify all *Salmonella* isolates with decreased susceptibility to fluoroquinolones.

Evidence from fluoroquinolone MIC distribution curves, from studies of clinical and bacteriologic response rates, and from PK/PD data, suggests that the current NCCLS fluoroquinolone breakpoint for resistance needs to be reevaluated for *S. enterica* serotypes and that further research is needed to guide the reevaluation process. The implications of reclassifying a substantial proportion of *Salmonella* isolates as fluoroquinolone nonsusceptible are complex and far-reaching, because alternative classes of antimicrobial agents for extraintestinal salmonellosis may be expensive to purchase, inconvenient to administer, and less efficacious than are fluoroquinolones for nalidixic acid–susceptible infections [73, 78, 79].

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